IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Anton Wellstein	
Serial No: 09/880097	Attorney Docket No. 102728-P01-004
Filed: June 14, 2001	Art Unit: 1649
For: PLEIOTROPHIN GROWTH FACTOR RECEPTOR FOR THE TREATMENT OF PROLIFERATIVE, VASCULAR AND NEUROLOGICAL DISORDERS	Examiner: Daniel E. Kolker

MS RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 CFR 1.132 of Anton Wellstein

Sir:

I, Anton Wellstein, residing in Washington DC, hereby declare as follows:

1. I am the inventor of the above-mentioned application which teaches and claims, among other things, a pleiotrophin-interacting extracellular fragment of the tyrosine kinase receptor ALK. I am a Professor of Oncology in the Department of Pharmacology at the Georgetown University Medical Center. In this declaration, I discuss experiments conducted in my laboratory under my supervision. In Exhibit A, I further include a letter from the journal Nature related to the peer review of a manuscript describing experimental findings in my laboratory.

2. I am aware that the U.S. Patent Office has rejected claims 95-99, 101 and 104 as being allegedly inherently anticipated by the disclosure of an abstract by Aigner et al., Proceedings of the American Association for Cancer Research (March 1999) 40: 732 (#4833). The abstract discloses screening of "a phage-display library of human fetal brain cDNA using recombinant PTN as a bait." It further discloses that "a candidate receptor fragment was identified which is contained in the

extracellular domain of an orphan transmembrane tyrosine kinase receptor." The abstract also states that "the receptor can be detected in 7 of 18 human breast cancer cell lines, in about 50% of all breast tumors and in different tumors of the CNS including glioblastoma and neuroma . . . ."

3. Based on the experience of researchers in my laboratory including myself, the phage-display screening for a PTN receptor involved many variables, none of which is mentioned in the Aigner et al. abstract. First, several phage-display libraries were available and tested in my laboratory, and only with one of them (the EasyMatch Phage Display human fetal brain cDNA library) were we able to identify ALK as the PTN receptor. Second, different phages including M13, Lamda, T7, or their modifications could be used in such a screening experiment, and not all of these options will yield a results. Third, recombinant PTN could be prepared by a number of methods published by us previously, and we found that only a biologically active PTN preparation could lead to successful screening of the phage-display library. Fourth, the quantity of bait PTN was a critical variable in reducing non-specific binding or noise in the screening experiments, and we found that only at an extremely high quantity (about 1 μg per well of a 96-well plate), the biologically active bait PTN could lead to the identification of an ALK fragment. Fifth, the panning itself also involved many variables such as incubation and wash conditions. After many experiments, we found that including Tween in the incubation and wash solutions was important in reducing non-specific binding. We also tested numerous blocking conditions to reduce background. Sixth, even with optimized panning conditions, biologically active bait PTN at a sufficiently high quantity, and the EasyMatch Phage Display library, we obtained numerous false positive results. In fact, the postdoctoral fellow, Sunitha Iruvanti, who conducted the panning experiments, and I disagreed on which screening results were true positives. Finally, the screening results obtained by the phage-display method represented only small peptide fragments, and the sequences of those small fragments had to be searched against sequence databases to identify the respective full-length proteins. Due to the small size of the screened peptide fragment, I personally had to vary many different parameters (such as BLOSSUM values) for running the NCBI BLAST search in order to identify a full-length protein that is homologous to the screened peptide fragment. The Aigner et al. abstract does not mention any of these variables. Given the difficulties we experienced, it is unlikely that another research group conducting a phage-display screening following the bare teachings of the Aigner et al. abstract would identify any PTN receptor, let alone ALK in particular.

4. Many transmembrane tyrosine kinase receptors had been identified by March 1999, and several had been known as orphan receptors. For example, HER2 had been well known as a ligand-less orphan tyrosine kinase receptor overexpressed in certain breast cancer cell lines or breast tumors. HER2 had also been known to express in tumors of the CNS such as glioblastoma. Therefore, another researcher in the field could interpret the orphan receptor disclosed in the Aigner et al. abstract as HER2 to name just one example.

5. Exhibit A includes a letter dated February 1, 1999 from the journal Nature that informed me of the negative peer review of our manuscript detailing the phage-display screening experiments that led to the identification of ALK as a pleiotrophin receptor. Exhibit A further includes a copy of one of the two peer reviews, which states that our experimental results were "rather unconvincing." Thus, even with detailed description of our phage-display screening experiments and follow-on characterization of ALK as a pleiotrophin receptor, some expert reviewers of the top-rated research journal Nature and researchers in the field did not appreciate our findings then.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: July 25, 2006 Signature: _____

Anton Wellstein, M.D., Ph.D.